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# Tree Breeding at the University of Tennessee 1959-1975

University of Tennessee Agricultural Experiment Station

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By Eyvind Thor



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## SUMMARY

Over the last 15 years a selection breeding program for forest trees has been in operation at The University of Tennessee. The general lack of basic information needed in breeding programs, such as data on geographic variation and heritability, required large investments in provenance and progeny tests. Results from these tests indicate that significant gains in wood production may be obtained from breeding programs with eastern white pine (*Pinus strobus*), Virginia pine (*P. virginiana*), loblolly pine (*P. taeda*), and yellow-poplar (*Liriodendron tulipifera*).

Breeding programs have also been established for three Christmas tree species: Fraser fir (*Abies balsamea* var. *fraseri*), Scotch pine (*Pinus sylvestris*), and Norway spruce (*Picea obies*). Significant progress has been made in selection of populations with the most suitable Christmas tree characteristics. Individual trees with good breeding potential—such as good needle-holding in Norway spruce—have been selected and production of both seed and cuttings has started.

Two long-term research efforts—one applied and one basic—now offer some hope that an American chestnut (*Castanea dentata*) with resistance to blight (*Endothia parasitica*) may be developed. A number of American chestnut trees with apparent resistance were selected and propagated in a clonal orchard. Evaluation of open- and control-pollinated progenies from this orchard has been initiated. Basic research efforts are concentrated on the chemical basis for resistance to the blight; some chemical fractions of inner bark extracts reduce in vitro growth of the blight fungus. Progress has been made in determining the chemical structure of the fungistatic compounds.

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## INTRODUCTION

The purpose of tree breeding is to develop trees with characteristics needed by man as raw material for industrial production or improvement of the environment. Two related terms are frequently used in connection with tree breeding: tree improvement and forest genetics.

Tree improvment refers to the developmental aspects of tree breeding, such as establishment and management of seed orchards, while forest genetics is a term which should be reserved for the research efforts carried out to supply the tree breeder with needed basic information. A tree breeder may attempt to cover the whole field from generating basic information to management of orchards, but as this field has expanded the individual workers have tended to specialize in either basic and applied research or developmental programs.

Tree breeding as a profession has, over the last 15 years, grown from a small group of forest scientists with missionary zeal to a few hundred scientists who have produced an impressive body of knowledge and an equally impressive gain in forest production. Of course, such rapid progress could not have been obtained if foresters had operated in a vacuum. We have taken advantage of knowledge accumulated over hundreds of years by agronomists, animal breeders, and horticulturists. Valuable inputs are continuously received from scientists in experimental statistics, computer science, botany, organic chemistry, pathology, entomology, and genetics. Outstanding scientists in all these fields are working for the Agricultural Experiment Station or other departments within The University of Tennessee system; many have given their support to the tree-breeding program.

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The Forestry Field Stations operated by the Agricultural Experiment Station have made it possible to establish breeding orchards and progeny tests across the state. Thus, a large amount of information and much valuable breeding material have accumulated, using a relatively small tree-breeding staff consisting of one senior scientist, one senior laboratory technician, two half-time research assistants, and one field man.

Cooperative work has also been carried out with agencies outside The University of Tennessee and with private land owners. Such efforts are aimed at developing trees with superior breeding value. When such trees have been identified, the next step is to establish seed orchards for production of commercial quantities of improved seed. This work is not research, but rather development, and in Tennessee it is taken care of by the State Division of Forestry. This organization has established a number of orchards; some of their loblolly pine and yellow-poplar orchards are already producing seeds which are used for production of seedlings in their nursery.

Plans for a tree-breeding program within The Agricultural Experiment Station were developed during the fall of 1959. The most urgent problem to solve was the selection of species to include in the breeding program. In addition to the more than 50 economically-important species native to Tennessee, the potential of exotic species, particularly conifers, could not be overlooked.

A number of factors entered into this decision; unfortunately, much information about these factors was lacking or incomplete. Thus, some assumptions had to be made based on educated guesses. It was decided that yellow-poplar was the native hardwood showing most promise for genetic improvement, and loblolly pine, white pine, and Virginia pine were the most promising conifers. Even though tree improvement efforts were centered on these four species, a series of species comparison tests were established so that adjustments in the breeding program could be made at a later date if other species were found to deserve greater attention than those initially selected. After 15 years of testing there is no indication of a need for a major revision of species in the program.

There are only six pine species native to Tennessee. However, it was recognized that some members of this important genus may have a high growth potential. Although exotics were not included immediately in the breeding program, it was decided to evaluate as many pine species as possible in an arboretum. Then, if any species showed great potential after 15 to 20 years, it could be added to the breeding program. The first species were planted in the Pinetum at the Highland Rim Forestry Field Station near Tullahoma in 1962. To date a total of 39 species have been planted in 1-acre plots. Only 18 species survived; none of the 16 species native to Mexico or the western United States had acceptable survival after 10 growing seasons. The 17 species which have survived in the Pinetum for more than 10 years are ranked (Table 1) for their potential according to growth rate. Species native to the southeastern United States have shown the best height growth indicating that we need not go outside of this region to select our breeding material of pine.

Forest trees have other uses than for timber. Aesthetic and wildlife values may, on some forest properties, be of greater importance than logs for lumber or paper. A species which is of particular interest in this respect is the American chestnut which once was the dominant tree in many Tennessee forests (1). In addition to providing lumber for farm buildings and wood extracts for tanning hides, the nut was a valuable source of food for both man and beast. Even though the complexity of a breeding program for blight resistance in American chestnut is discouraging, the importance of this species to the people of Tennessee warranted such an effort.

For many small landowners in Tennessee, the planting of trees for timber production does not have great appeal because it takes so many years before timber can be harvested and a profit realized. As a result, the possibility of growing Christmas trees on an 8-10 year rotation was investigated. Data from this study indicated that there was a good market for locally-grown trees (2); also, the growing conditions in East Tennessee appeared to be well suited for producing several acceptable species (3). Nine of the most promising species were tested in several locations in East Tennessee. At the lower elevations (less than 2,000 ft.), Norway spruce (*Picea abies*), blue spruce (*P. pungens*), white spruce (*P. glauca*), and white pine (*Pinus strobus*) grew well and about 80 percent were graded as premium quality trees (4). On poorer sites, Scotch pine (*Pinus sylvestris*) performed well and on good sites at high elevations (over 3,000 feet) Fraser fir (*Abies balsamea* var. *fraseri*) appeared to have great potential. These tests have served as an excellent guide in planning our breeding efforts with Christmas trees. Not only did we determine which species had the greatest potential, but the amount of variation observed among trees within a species gave a strong hint as to which species would respond best to a selection breeding program.

## TIMBER SPECIES

### White Pine

Eastern white pine (*Pinus strobus*) is not the most important timber species in Tennessee; it ranks behind three southern pines and several hardwood species with respect to growing stock volume. However, about 2 million white pine seedlings are planted each year,

TABLE 1. Mean annual height growth for 17 pine species which have survived in the Pinetum for 10 years.

| Pinus spp. | Common name | Annual height growth, feet |
|---|---|---|
| *taeda* | loblolly | 3.0 |
| *serotina* | pond | 2.5 |
| *elliottii* | slash | 2.4 |
| *glabra* | spruce | 2.2 |
| *palustris* | longleaf | 2.1 |
| *pungens* | table mountain | 2.0 |
| *virginiana* | Virginia | 2.0 |
| *echinata* | shortleaf | 1.9 |
| *rigida* | pitch | 1.8 |
| *strobus* | eastern white | 1.6 |
| *clausa* | sand | 1.6 |
| *sylvestris* | Scotch | 1.5 |
| *banksiana* | Jack | 1.4 |
| *thunbergii* | Japanese black | 1.3 |
| *densiflora* | Japanese red | 1.3 |
| *pinaster* | maritime | 1.2 |
| *nigra* | Austrian | 1.2 |

a number of them for production of Christmas trees and landscaping material.

Even though white pine wood is desirable for certain specific purposes, the low specific gravity and associated poor strength in lumber and low yield for pulp are reasons why this species has not been planted on a wider scale. Another important reason is that white pine is very susceptible to damage from air pollution. A number of coal-fired steam plants in East Tennessee emitting large amounts of sulfur dioxide have been held responsible for the killing or damaging of forest stands and Christmas tree plantations. Thus, important objectives of a white pine breeding program would include an evaluation of the variation in wood properties and resistance to air pollution.

A study of variation in wood properties confirmed that the specific gravity of white pines in the Cumberland Mountains was low (about 0.41). However, individual trees were found with a density of 0.47 indicating that progress may be made in a selection breeding program (5). This conclusion was supported with data from a 25-

year-old plantation, which suggested that no more than 24 percent of the variation in wood specific gravity can be accounted for by site variables (6).

The amount of extractives obtained by alcohol and benzene extraction was surprisingly large, amounting to about 8 percent. Some individual trees had as much as 15 percent of their weight in extractives. Since many of the extractives, such as the monoterpenes, have great commercial value, a study was designed to determine the variation and inheritance of monoterpene composition. The yield of turpentine—basically alpha- and beta-pinene—was about 22 percent of the oleoresin (7). An opportunity exists to increase this yield by breeding; variation among clones is exceptionally great and the broad sense heritability is high (H=0.66).

The low wood density of white pine, and resulting low pulp yields, has in the past made it unprofitable to thin young stands. Without thinnings the trees grow slowly and sawtimber rotations may become too long for a profitable investment. The recent finding that paraquat, a herbicide, will cause resin soaking in pine stems may provide a partial answer to the problem of utilizing small stems. A combination of breeding and chemical treatment of trees offers promise that we may in the future control both the quantity and quality of extractives in white pines.

A more immediate problem confronting the researcher is to breed trees which will grow well at the high sulfur dioxide levels prevalent in Tennessee. Fortunately, there is much genetic variation in the susceptibility of white pines to air pollution. In stands which have been destroyed by high levels of sulfur dioxide, individual trees have been found with no apparent damage. That this resistance is genetically controlled has been confirmed in clonal tests (Figure 1).

Since white pines for most uses are propagated by seed (sexually) rather than by cloning (asexually) it is also necessary to determine the narrow sense heritability. Two progeny tests, one in the fall-out area from Kingston Steam Plant, the other next to Bull Run Steam Plant, are giving this information. Open-pollinated families representing 90 mother trees from the Southern Appalachians have completed six growing seasons in the field and are being evaluated for symptoms of air pollution (yellow, short needles and tip burn). In the Oak Ridge plantation the progenies from stands in Anderson, Morgan, and Scott counties, located in the airsheds of the two steam plants, had longer, darker-green needles than progenies from other stands in the Southern Appalachians (Table 2). Also, trees from Polk and Monroe counties in Tennessee and Fannin County in Georgia had a high "needle index" indicating that the proximity of these stands to the Copperhill smelting has resulted in removal of the most susceptible genotypes from these populations.

FIGURE 1. After 10 years grafted white pines demonstrate great differences in resistance to damage from $SO_2$ - pollution. The ramets to the left came from a highly susceptible tree; most have been killed by pollution from a coal-fired steam plant. Ramets in the middle row have some resistance while the vigorous trees to the right are ramets of a highly-resistant clone.

To get a better estimate of the variation of white pine in the Southern Appalachians, a plantation containing progeny from 26 stands was established on the Highland Rim near Tullahoma. Collections had been made from the southernmost part of the species range in Georgia (Latitude 34°43' N) to West Virginia (Lat. 39°30' N). After five growing seasons in the field, the trees from the three best sources were significantly taller (4.81 feet) than those from the eleven poorest (3.07 feet). All but one of the 11 poor sources came from Virginia or West Virginia; apparently, in Tennessee we

TABLE 2. Ranking of 13 white pine stands according to needle color and needle length of 5-year-old progenies growing near Bull Run steam plant.

| Stand No. | Stand Location | Color Score a) b) | Needle Length, cm. | Needle index c) |
|---|---|---|---|---|
| 13 | Anderson Co., Tenn. | 3.07 | 7.52 | 3.28 |
| 11 | Scott Co., Tenn. | 3.03 | 7.50 | 3.22 |
| 2 | Polk Co., Tenn. | 3.00 | 7.31 | 3.00 |
| 3 | Fannin Co., Ga. | 2.94 | 7.00 | 2.63 |
| 12 | Morgan Co., Tenn. | 2.85 | 7.81 | 3.35 |
| 1 | Monroe Co., Tenn. | 2.73 | 7.18 | 2.60 |
| 8 | Unicoi Co., Tenn. | 2.70 | 7.08 | 2.47 |
| 5 | Madison Co., N.C. | 2.64 | 7.07 | 2.40 |
| 4 | Cherokee Co., N.C. | 2.52 | 6.81 | 2.02 |
| 10 | Carter Co., Tenn. | 2.36 | 7.32 | 2.36 |
| 7 | Unicoi Co., Tenn. | 2.30 | 7.13 | 2.11 |
| 6 | Cocke Co., Tenn. | 2.22 | 7.00 | 1.91 |
| 9 | Transylvania Co., N.C. | 1.62 | 5.69 | 0.00 |

a) Each tree was rated 1 for greenish-yellow, 2 for yellow-green, 3 for bluish-green, and 4 for greenish-blue.

b) Vertical lines connect sources not significantly (5 percent level) different in color rating according to the New Duncan's Multiple Range Test.

c) Combined value for needle color and needle length.

can insure ourselves against poor seed lots by excluding such "Northern" imports. A regression analysis indicated that 45 percent of all seed source variation in height was accounted for by latitude (8). This relationship of progeny height growth to latitude of origin is presented in Figure 2. Even though the negative correlation coefficient (r=-0.67) reflects the propensity of the data points to lie in a band extending from the upper left to the lower right, there are—especially at the more southern latitudes—large deviations from the regression line.

Since so much variation was unaccounted for by latitude, additional variables—such as elevation and number of frost-free days of the source—were added in a multiple regression analysis. However, the equations derived did not account for a significantly greater amount of the variation than the simple regression equation with latitude as the only independent variable. The wide variation in height growth of trees from different stands at the extreme southern end of the species range suggested that more intensive sampling of stands from this part of the species range would be necessary to capitalize on the apparent ecotypic variation.

One such study was established in 1970 with open-pollinated progenies from 13 stands in Georgia, North Carolina, and Tennes-

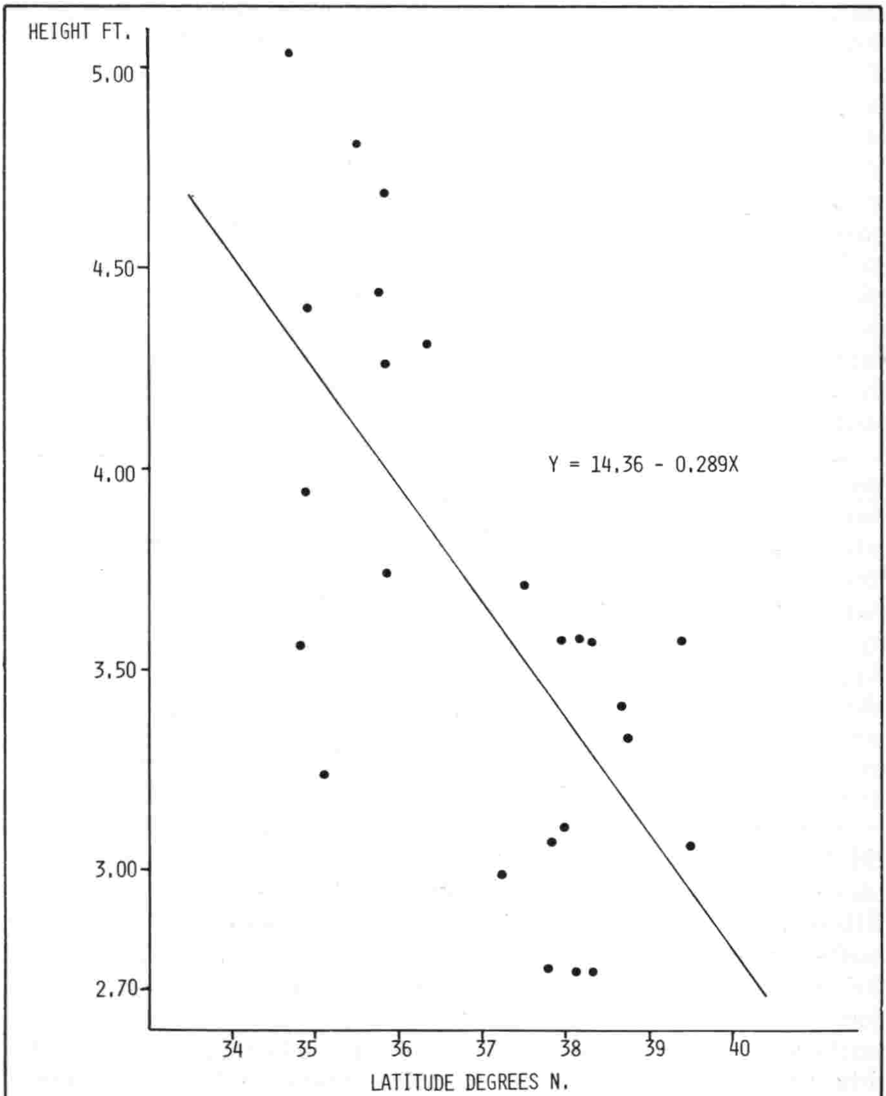


FIGURE 2. After five growing seasons in the field, white pines from southern latitudes were much taller than those of more northern origin.

see. A total of 42,600 two-year old seedlings representing 129 open-pollinated families were planted in four physiographic regions of Tennessee (West, Highland Rim, Cumberland, and Great Valley). Five-year height measurements have been obtained from three of these plantations.

Results from the stand analysis strongly support the conclusion from our previous study; differences in growth rate of trees from different stands in the Southern Appalachians were highly significant. Trees from the stands in Anderson, Morgan, and Scott counties consistently grew fastest in the three test locations (Table 3). On the Highland Rim, progenies from these three stands grew 25 percent faster than the progenies from the three North Carolina stands, and a superiority of 28 percent was recorded for the Great Valley test site in Anderson County. However, these differences in growth between North Carolina and local sources were small compared to differences observed at the Cumberland Mountain test site in Morgan County; in this location trees of local origin (Anderson, Morgan, and Scott counties) had a height superiority of 53 percent over those from North Carolina. Differences of this magnitude indicate that in addition to the north-south clinal variation pattern, there has been considerable ecotypic differentiation in the extreme southern part of the species range.

Knowledge of the clinal variation pattern has been available for several years and foresters, both in this country and Europe, have purchased white pine seed from the Southern Appalachians to take advantage of the rapid growth rate associated with this source. Results have, however, often been poorer than anticipated and variation in success can probably to a large extent be accounted for by genetic variation among populations in the Southern Appalachians. The superiority of the sources from Anderson, Morgan, and Scott counties may be limited to plantations in Tennessee; other states and foreign countries must do their own testing to establish which ecotypes are best suited for their growing conditions.

Ten to 15 percent of the total variation in height observed in individual plantations was accounted for by stands (source of seed). An additional 5 to 7 percent of variation in height was contributed by differences among half-sib families. Within most stands there were mother trees which produced progeny that grew significantly faster than progeny from other mother trees in the same stand. This variation among families is reflected in the estimates of narrow sense heritability, varying from 0.20 to 0.33. By selecting the best individual progenies from the best families, it is possible to make gains of about 20 percent in juvenile height growth. Such gains may, of course, be added to any gains already obtained by using seed of the most suitable ecotypes. Thus, the minimum gain in juvenile height

TABLE 3. Height in feet of white pines from 13 stands after five growing seasons in three locations.

| Stand No. | County | State | Great Valley | | Cumberland Mt. | | Highland Rim | |
|---|---|---|---|---|---|---|---|---|
| | | | Height, ft. | Rank | Height, ft. | Rank | Height, ft. | Rank |
| 1 | Monroe | TN | 4.44 | 10 | 5.68 | 9 | 5.47 | 10 |
| 2 | Polk | TN | 4.71 | 7 | 6.65 | 4 | 5.74 | 6 |
| 3 | Fannin | GA | 4.74 | 6 | 5,81 | 7 | 5.72 | 7 |
| 4 | Cherokee | NC | 3.92 | 12 | 4.66 | 12 | 4.86 | 11 |
| 5 | Madison | NC | 4.69 | 8 | 5.07 | 11 | 5.52 | 8 |
| 6 | Cocke | TN | 4.51 | 9 | 5.80 | 8 | 5.52 | 9 |
| 7 | Unicoi | TN | 4.83 | 3 | 6.29 | 5 | 5.82 | 4 |
| 8 | Unicoi | TN | 4.80 | 5 | 6.20 | 6 | 5.78 | 5 |
| 9 | Transylvania | NC | 3.59 | 13 | 4.27 | 13 | 4.63 | 13 |
| 10 | Carter | TN | 4.10 | 11 | 5.16 | 10 | 4.71 | 12 |
| 11 | Scott | TN | 4.81 | 4 | 6.75 | 3 | 6.02 | 3 |
| 12 | Morgan | TN | 5.14 | 2 | 7.34 | 2 | 6.29 | 2 |
| 13 | Anderson | TN | 5.63 | 1 | 7.36 | 1 | 6.52 | 1 |
| Mean | | | 4.72 | | 6.05 | | 5.63 | |

growth from one generation of selection will be about 20 percent (assuming that the best seed sources already were used) while the maximum gain will be between 45 and 73 percent (assuming that the poorest Southern Appalachian sources had previously been planted). Such large differences in height growth are illustrated in Figure 3.

FIGURE 3. Open-pollinated progenies from a white pine in Cocke County only average 6 feet in height after six growing seasons while progenies from an Anderson County tree are 10 feet tall.

## Yellow-Poplar

Yellow-poplar (*Liriodendron tulipifera*), a magnificent tree which is desired in many industrial processes, is also the state tree in Tennessee. Studies of variation in wood properties indicated that much phenotypic variation was present for density (wood specific gravity), but variation in fiber length and amounts of wood extractives were relatively minor (9). Using research data and general observations a selection index was developed which has been used to select nearly 100 superior phenotypes in Tennessee.

Two basic assumptions were made in making these selections: that the characteristics used in the index were strongly inherited and that the local populations (stands) were as good as or better than stands in other parts of the species range. To test the validity of these two assumptions, several progeny tests and provenance tests have been established.

Differences among sources are usually apparent in the relatively uniform environment of a nursery bed. However, a few years after outplanting in the field, differences often cannot be detected as statistically significant because site variation within replications results in large error terms. Most tree breeders learned their trade working with pines, and minor site variation within replications caused few problems with species relatively insensitive to minor site changes. Yellow-poplar, however, is exceedingly site-sensitive, and height growth may change dramatically with almost imperceptible changes in site conditions. We try to solve this problem by using more uniform land, uniform control of competing vegetation, fertilization to obtain uniformly high fertility levels, and small plots (3-4 trees) to reduce block size and permit a much greater number of replications.

Fifteen years ago yellow-poplar seed source tests were established on former agricultural sites at five locations in Tennessee (Figure 4). A total of six seed sources were included in this test, but only four were used in any one plantation. All sources were from Tennessee or bordering counties in North Carolina, Georgia, and South Carolina. A great advantage in height of the trees growing on the good, uniform agricultural site in the East Tennessee Valley was established over the first ten growing seasons. After 15 years this plantation was still the most uniform with no significant differences among replications. The Cumberland Plateau plantation was also on a relatively uniform site, but the other three planting sites were highly variable. Statistically significant differences among seed sources were only found on the two uniform planting sites (10).

In the East Tennessee Valley plantation trees from relatively high elevations in western North Carolina grew significantly slower than those from a low elevation in West Tennessee. The West Tennessee trees averaged 48 feet in height and 6 inches in diameter after

FIGURE 4. Yellow-poplar test plantations have excellent growth on good agricultural sites.

15 growing seasons while the North Carolina trees were only 39 feet tall with a diameter of 4.4 inches. The average West Tennessee tree produced 4.3 cubic feet of wood while the average tree of North Carolina origin grew less than half that much (2.0 cubic feet). On the Cumberland Plateau trees originating from the Cumberland Mountains of Tennessee had poorer growth than those from the other three sources. After 15 years the trees from this high elevation source were only 20 feet tall and averaged 2.3 inches DBH, while the other three sources averaged 27 feet in height and 3.3 inches in diameter. Volume growth of the Cumberland Mountain trees was only 0.3 cubic feet, compared to 0.8 for those from the other three sources.

Even though all the seed sources used in these tests were "local" and could have been recommended by prudent foresters, trees from two of the sources have in specific locations produced less than half as much wood as the trees from the best adapted sources. In both plantations it was the source from the highest elevation which performed most poorly. Although no generalization may be made from these limited tests, the results indicate that studies of altitudinal variation in yellow-poplar are needed.

To establish breeding orchards which would produce flowers within a reasonably short period of time it was necessary to graft scion wood from the upper crown of mature yellow-poplars onto seedling understock (11). We soon learned that timing of this operation was important; better survival of grafts was obtained by grafting in mid-April (12). Some ramets produced a few flowers 2 to 3 years after establishment; however, large amounts of seed were only obtained after 10 years. When enough clones had started to flower, controlled crosses were made (Figure 5) and the resulting progenies outplanted.

Two types of statistical analysis were carried out on height data obtained after one and four growing seasons. The first type of analysis gave estimates of both additive and dominance variances. After 1 year the dominance component was highly significant, but the additive component was not. After 4 years in the field the dominance component was still significant, but no additive effect could be detected. In the second analysis, 34 crosses plus four check sources were included. Duncan's multiple range test revealed that height means fell into three groups consisting of approximately an upper quarter, a middle half, and a lower quarter. After four growing seasons, most of the progenies in the upper quarter had Number 2 or 10 as male or female parent; both these clones originated in East Tennessee. The commercial check progenies ranked near the bottom of the list; but two crosses with Clone 10 and one with Clone 2 were also inferior, confirming that no claims for good general combining ability should be made for these two clones.

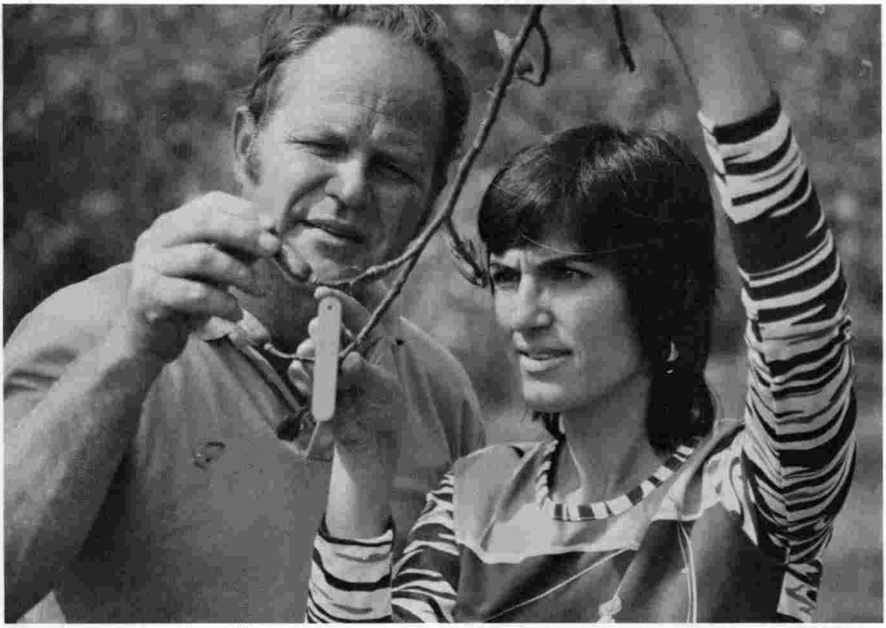

FIGURE 5. Professor Thor and senior research assistant Safiya Samman examine mature yellow-poplar "cones" (aggregates of samaras) resulting from control-pollination in the breeding orchard.

Although these data were obtained from a limited number of crosses in a single progeny test plantation, there is a strong indication that specific combining ability for height growth may be present. If the more comprehensive tests now being established yield similar results we must change our breeding procedures to take advantage of the specific combining ability. Since the best crosses, after 4 years in the field, were 50 percent taller than the poorest, crossing and testing should be continued to determine which combinations produce seed with good germination and vigorous seedlings.

Only about 10 percent of most open-pollinated samaras contain a viable seed. Since each samara has the potential for two seeds, actual viability is seldom more than 5 percent. In plantation management, low seed viability results in expensive cone collection. Even though there are as many as 150,000 samaras per bushel of cones, less than 15,000 seeds will normally germinate in the nursery bed.

Fortunately, there is much genetic variation among individual trees in seed viability. Samaras from the grafted orchard near Knoxville have been examined for several years using radiography; the poorest clone usually has about 2 percent filled seed, while the best clone has 27 percent. Hand pollination to insure crossing tends to increase seed viability (13). When 15 clones were used as female parents, the resulting samaras had a viability ranging from 5 to 35 percent. However, when the same clones were used as pollen parents, the range was only 10 to 19 percent; this result indicates that the viability level of a given cross is primarily controlled by maternal factors (14). By selecting clones for the orchard which consistently produce a high proportion of filled seed it is possible to market genetically improved seed which will have additional value because of superior germinative capacity.

Insects are the principal pollinating agents and it has been suggested that with proper management of domestic beehives a higher pollination rate may be obtained. Such a program was started in our yellow-poplar orchard near Knoxville. One hive was provided early

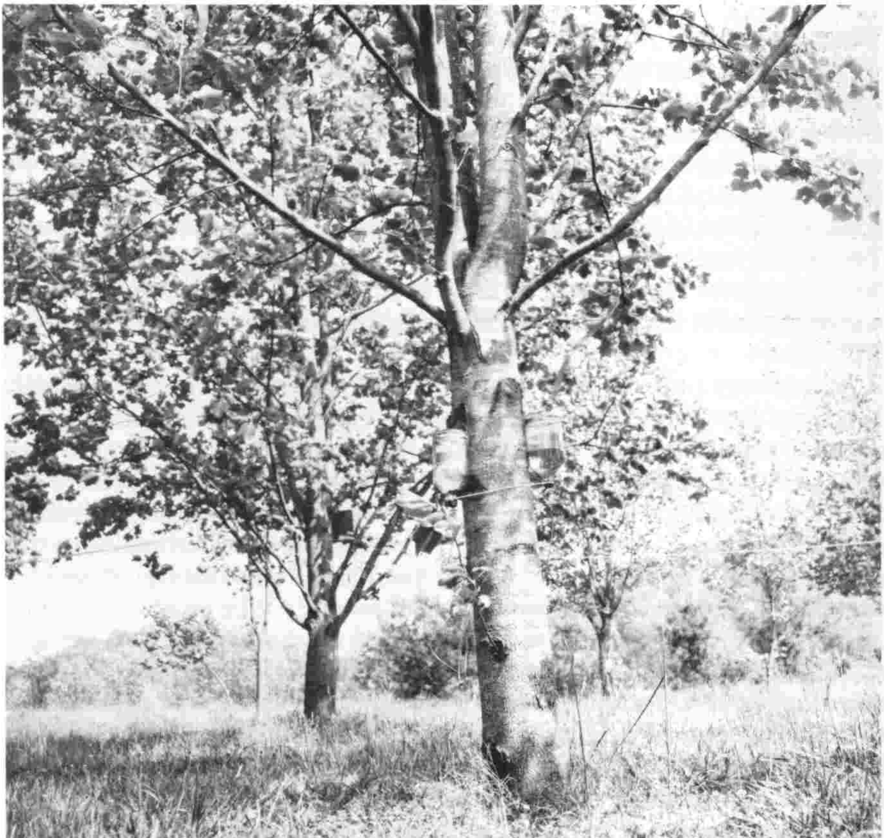

FIGURE 6. Radioactive phosphorus introduced into the stem was taken up by the flowers. Bees carried radioactive pollen from this source tree to orchard trees as far away as 400 feet.

pollen supplements so that it was at full strength at the time of flowering. The hive was placed adjacent to a large orchard tree having hundreds of flowers tagged with radioactive phosphorus (Figure 6). No radioactive pollen was found in the hive, indicating that the numerous bees visiting this tree came from hives some distance from the orchard. These stray bees carried radioactive pollen from the source tree to orchard trees as far away as 400 feet (15). Thus, honeybees may be efficient in providing cross pollination needed for obtaining yellow-poplar seed with high viability; however, we need to know more about managing bees to maintain high concentrations within the orchard.

By using clones in the orchard which have good combining ability for production of filled seed and managing bees to insure adequate pollination we expect to increase seed viability to 20 percent and thus cut seed collection costs in half.

**Virginia Pine**

Although Virginia pine (*Pinus virginiana*) is considered a minor species among the southern pines, it has wide distribution in Tennessee and makes up about one-third of all coniferous growing stock volume in the state. Virginia pine invades abandoned and eroded farm lands and is capable of producing a timber crop even on very poor, dry sites. Also, thousands of acres of mountain land in East Tennessee are strip-mined for coal; these lands will, according to law, have to be reclaimed and it is expected that Virginia pine will be one of the most important species in reclamation work on acid spoils (16).

Since very little information was available about the variation in Virginia pine, a study was designed to determine the variation in natural populations and in open-pollinated progenies from mother trees selected in these populations. In 1962 the Kentucky-Tennessee Section of the Society of American Foresters decided to support such a study and Section members cooperated in locating suitable stands and assisted in obtaining data. Fifteen trees were sampled within each of 13 stands in Tennessee and Kentucky. For most characteristics measured there were highly significant differences among stands (17). However, the wide range in stand averages for wood specific gravity before extraction (from .49 to .55) was drastically reduced by removal of the extractives (.47 to .51). Apparently, the large variation in specific gravity of unextracted wood are partly caused by the extractives which have large variation among stands.

The variation among trees within stands was highly significant for all the characteristics measured, and a large proportion of the total variation was accounted for by the among-tree variance component (Table 4). One cannot be sure how much of this individual tree variation is due to environmental factors, but since the trees

TABLE 4. Components of variance (in percent) for radial growth and wood properties of Virginia pine

| Characteristics | Stands | Trees in stands | Samples within trees |
|---|---|---|---|
| Radial growth | 21 | 47 | 32 |
| Sp. gr. before extraction | 14 | 57 | 29 |
| Extractives | 29 | 38 | 33 |
| Sp. gr. after extraction | 4 | 68 | 28 |
| Tracheid length at 10 years | 32 | 56 | 12 |
| Tracheid length at 25 years | 46 | 45 | 9 |

within stands were growing under relatively uniform conditions, it is difficult to see how these great differences could be due to environmental effects alone.

Seed was collected from most of the sample trees and 1-year-old progenies were outplanted in four locations in Tennessee: West Tennessee (A), Highland Rim (B), Cumberland Plateau (C), and the Great Valley (D). In addition, plantations were established in North Alabama (E) and on a reclaimed strip-mine site in East Kentucky (F). A total of about 60,000 trees were planted, representing 128 of the trees sampled in the study of natural variation. These plantations have been evaluated after two and five growing seasons in the field (18, 19). Due to the large amount of data and many sources of variation and interactions, rather complicated statistical analyses had to be completed (20). These analyses made it possible to obtain estimates of the variance components needed to determine the heritability of growth rate and wood specific gravity and to estimate the expected gains from selection breeding programs.

Of the 13 stands originally sampled, only 10 yielded enough seedlings to plant in all six locations. Mean heights for stands after 2 and 5 years and their ranking at each test site are given in Table 5. The Highland Rim site (B) has the overall largest heights after both two and five growing seasons while the smallest heights were observed on the poor strip-mine site in Kentucky (F). However, regardless of site conditions, the trees from stand number 10 (near Etowah) were the fastest growers. The other two stands representing the Great Valley, number 11 and 13, also ranked near the top indicating that seed for planting in Tennessee should be collected from this physiographic region. When the plantations were 5 years old, they were measured for stem diameter in addition to total height so that stem volume (in cubic feet) could be determined for each tree. Data

TABLE 5. Ranking[a] of Virginia pine stands within each test site and mean heights at 5 years

| Rank a) | Test sites: A | | B | | C | | D | | E | | F | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Stand no. | Height ft. | Stand no. | Height ft. | Stand no. | Height ft. | Stand no. | Height ft. | Stand no. | Height ft. | Stand no. | Height ft. |
| 1 | 10 | 8.9 | 10 | 10.5 | 10 | 9.2 | 10 | 9.4 | 10 | 8.4 | 10 | 5.5 |
| 2 | 11 | 8.3 | 13 | 10.3 | 7 | 9.1 | 13 | 9.2 | 13 | 8.0 | 11 | 5.4 |
| 3 | 13 | 8.3 | 4 | 10.2 | 9 | 8.9 | 7 | 8.8 | 11 | 7.7 | 13 | 5.2 |
| 4 | 7 | 8.2 | 11 | 10.2 | 13 | 8.8 | 9 | 8.7 | 4 | 7.7 | 7 | 5.2 |
| 5 | 4 | 8.1 | 9 | 10.0 | 8 | 8.8 | 11 | 8.7 | 7 | 7.7 | 9 | 5.1 |
| 6 | 6 | 8.0 | 7 | 10.0 | 11 | 8.6 | 8 | 8.7 | 8 | 7.6 | 3 | 5.1 |
| 7 | 8 | 7.9 | 6 | 9.9 | 4 | 8.6 | 4 | 8.6 | 6 | 7.5 | 4 | 4.8 |
| 8 | 9 | 7.8 | 8 | 9.8 | 6 | 8.5 | 3 | 8.5 | 9 | 7.4 | 2 | 4.8 |
| 9 | 3 | 7.7 | 3 | 9.7 | 3 | 8.4 | 6 | 8.4 | 3 | 7.4 | 6 | 4.7 |
| 10 | 2 | 7.6 | 2 | 9.4 | 2 | 7.9 | 2 | 8.0 | 2 | 7.2 | 8 | 4.6 |
| Mean | | 8.1 | | 10.0 | | 8.7 | | 8.7 | | 7.7 | | 5.0 |

a) **Stands ranked in descending order by stand mean heights.**

analysis combined for all planting locations indicated that the family-within-stand component accounted for as much of the variation in stem volume as the among-stand component (4.6 percent of total phenotypic variation). The heritabilities for stem volume (Table 6) indicated that progress in breeding for tree volume could be made by individual tree selection as well as by selection of stands (Figure 7). Analysis of volume data combined for all plantations revealed that the interactions between genotypes and plantation location were so small that they could be ignored in a breeding program. Thus, it would not be necessary to develop separate seed orchards for the different physiographic regions of Tennessee; one orchard could produce seed that would be adapted to all planting sites in the state.

In each of five plantations the best individual progenies from the best families and stands were selected. The expected gains in stem volume at each location resulting from such three-stage selection are listed in Table 6. This scheme provides about 10 percent improve-

TABLE 6. Population means for five Virginia pine test plantations, narrow sense heritabilities and expected volume gains per tree at age 5, resulting from three-stage selection. Numbers in parentheses indicate percent contribution of each stage of selection

| | Test sites | | | | |
|---|---|---|---|---|---|
| **Component** | **A** | **B** | **C** | **D** | **E** |
| Mean tree volume, unselected population, cu. ft. | 0.081 | 0.151 | 0.089 | 0.090 | 0.114 |
| $h^2$ | 0.15 | 0.24 | 0.33 | 0.08 | 0.24 |
| Gain from stand selection, cu. ft. | 0.012 | 0.015 | 0.012 | 0.010 | 0.012 |
| | (12.6) | (9.9) | (13.3) | (11.1) | (10.8) |
| Gain from family selection, cu. ft. | 0.009 | 0.016 | 0.017 | 0.006 | 0.015 |
| | (10.5) | (10.8) | (19.3) | (6.3) | (13.3) |
| Gain from within family selection, cu. ft. | 0.010 | 0.022 | 0.026 | 0.005 | 0.023 |
| | (12.2) | (14.5) | (29.2) | (6.0) | (19.8) |
| Total gain, cu. ft. | 0.029 | 0.053 | 0.055 | 0.021 | 0.050 |
| Estimated mean of new population, cu. ft. | 0.110 | 0.204 | 0.144 | 0.111 | 0.164 |
| Percent gain, three-stage selection | 35.2 | 35.2 | 61.91 | 23.5 | 43.9 |

FIGURE 7. Large differences in volume growth among open-pollinated families of Virginia pine were evident following eight growing seasons in the field. Family 12-5 (to the left) came from a mother tree in the East Tennessee Valley; the progenies were, on an average, 20 feet tall and contained 1.72 cubic feet of stem wood. Family 2-8 (to the right) came from a mother tree in West Kentucky; the average progeny was only 16 feet tall containing 0.61 cubic foot of wood.

ment from stand selection; gains from family and within-family selection vary considerably from location to location. Since the trees were only 5 years old when measured, very little competition among individuals had taken place and a gain of 0.05 cubic foot per tree

(Table 6) may be equivalent to 50 cubic feet per acre (1,000 trees per acre). Assuming a stumpage of $5.00 per 100 cubic feet this gain will be worth $2.50 per acre over the first 5 years of the rotation. Volume gains over the next 20 years on an individual tree basis will undoubtedly increase, but such estimates may, to a large extent, be the result of competition among individuals and families and should not be used to estimate gain on a per-acre basis.

## Loblolly Pine

Although loblolly pine (*Pinus taeda*) is only native to a few of the southernmost counties in the state, more seedlings of this species are planted than all other forest trees combined. This popularity is due to fast growth on a great variety of sites and the desirable pulping properties of the wood.

Since most of Tennessee is located north and west of the natural range of loblolly pine, the first task in the breeding program was to determine the geographic variation pattern and the provenances best suited for Tennessee growing conditions. In a rangewide study of natural variation, many morphological characteristics were investigated (21). In one respect all these variables behaved alike; a large proportion of the total variation was accounted for by the tree-to-tree variation. Some characteristics which showed highly significant differences among locations did not show any geographic trend, but other characteristics gave good evidence of regional variation patterns. Large differences among stands from different parts of the species range and the variety of geographic patterns indicated the need for extensive seed source testing prior to the establishment of production seed orchards.

The primary objective of these tests was to pinpoint the physiographic regions where we could select superior phenotypes and be relatively certain that they were from populations well adapted to the growing conditions in Tennessee. The secondary objective was to establish which physiographic regions may be used for procurement of needed seed until seed orchards produce the required amounts.

Fortunately, a few loblolly pine provenance tests had been established just prior to 1960, mainly by TVA. Several additional tests were established and detailed measurements and analyses of all tests were carried out on a regular basis, usually every 5 years. Some of these tests have given more information than others; the selection of seed sources to be tested, experimental design, and site conditions have influenced the value of individual tests. However, when all the tests were evaluated, some definite trends emerged.

The oldest and best-designed test was established in 1956 in the Cumberland Mountains at 1,300 feet above sea level (22,23). Six seed

TABLE 7. Growth data and ranking of trees from six loblolly pine seed sources for 20 years in a Cumberland Mountain plantation

| Seed source | Two years | | Five years | | Ten years | | Fifteen years | | Twenty years | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Ht. ft. | Rank | Ht. ft. | Rank | Tons/A | Rank | Tons/A | Rank | Tons/A | Rank |
| Southeast Tennessee | 2.4 | 6 | 10.1 | 6 | 6.7 | 3 | 35.2 | 3 | 65.1 | 1 |
| Alabama Piedmont | 2.5 | 5 | 10.3 | 4 | 6.8 | 2 | 35.3 | 2 | 58.8 | 3 |
| North Georgia Piedmont | 3.0 | 2 | 10.7 | 2 | 5.0 | 6 | 26.1 | 6 | 42.7 | 6 |
| North Carolina Piedmont | 3.2 | 1 | 11.9 | 1 | 8.9 | 1 | 40.9 | 1 | 65.1 | 1 |
| North Carolina Coastal Plain | 2.5 | 4 | 10.2 | 5 | 5.8 | 4 | 33.3 | 4 | 58.1 | 4 |
| South Carolina Coastal Plain | 2.7 | 3 | 10.6 | 3 | 5.2 | 5 | 29.3 | 5 | 51.7 | 5 |

sources were included (Table 7). Differences among sources in height growth became apparent after 2 years in the field, but more dramatic differences were observed during the winter of 1962-63, when the temperature dropped below zero degree F. on three different days. The two southernmost sources, South Carolina Coastal Plain and North Georgia Piedmont, suffered the most needle damage. That these two sources are poorly adapted to Tennessee growing conditions was evident after 20 growing seasons when they ranked lowest in production (extracted dry wood in tons per acre) of the six sources. This plantation received a light low thinning at 15 years and is now, at 20 years, approaching the end of a pulpwood rotation containing some individual trees of sawlog size and quality. Based on evaluations after two and five growing seasons, one "winner" was picked; the North Carolina Piedmont source ranked first in every inventory. However, in the last inventory it shared this position with the Southeast Tennessee source, which ranked at the bottom in the first two inventories. This observation points out the danger of making decisions based on height growth in young plantations. After 20 years, there were no significant differences among sources for height; but for basal area and wood production the differences were significant, both in a statistical and economical sense. The two top sources produced 50 percent more wood than the poorest source.

Only by evaluating several seed source tests, such as the one described above, is it possible to make recommendations for the entire state. All 10 known seed source plantations in Tennessee have been evaluated (24); the results indicate that in most parts of Tennessee, loblolly pine originating from the Northeast part of the species range (Maryland, Virginia, and North Carolina) grow faster than those of inland sources and much faster than those from Deep South Coastal Plain sources (Figure 8).

The adaptability of loblolly pine for planting in Tennessee appears to be related to mean length of the freeze-free period. Trees of provenances with between 190 and 210 freeze-free days (Northeast sources) produced the most wood in all but the extreme southwest part of the state. Provenances with freeze-free periods between 210 and 240 days (inland sources) produce trees with intermediate growth, except in extreme southwest Tennessee where inland sources were best. In the southwest corner of Tennessee, the frost-free season is longer than in the rest of the state (more than 210 days), and inland sources may be better adapted to a longer growing season. Trees adapted to longer freeze-free periods (Deep South Coastal Plain sources) often suffered frost damage and generally yielded the poorest results.

These results formed the basis for the two following conclusions: Superior trees for loblolly pine seed orchards in Tennessee should

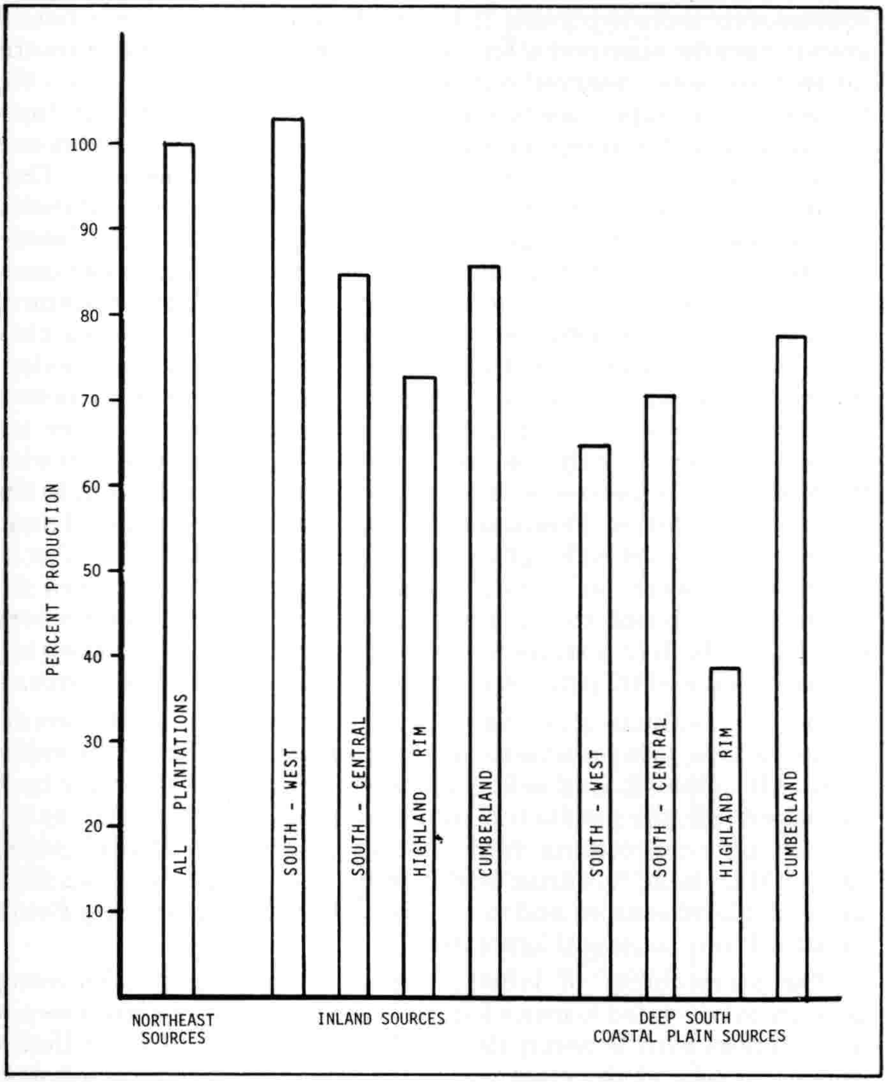


FIGURE 8. When the performance of loblolly pines of Northeast sources (Maryland, Virginia, and North Carolina) in all seed source plantations is considered 100 percent of productivity (measured in tons per acre), the Inland and Deep South Coastal Plain seed sources are generally much inferior. Only in the extreme southwest part of Tennessee trees of Inland sources (South Carolina Piedmont to Arkansas) produced more wood than trees of the recommended Northeast sources.

mainly be selected from the northeast part of the species range, while some selections from the northern part of the interior range also will be included; during the next few years, until our orchards produce sufficient amounts of seed, commercial seed lots should be restricted to areas North and East of Tennessee, except that seed for use in West Tennessee may be of northern inland sources from the South Carolina Piedmont to Arkansas.

At the start of the program with loblolly pine, a breeding orchard was established to serve two purposes; it should act as a clone bank where potentially valuable genotypes may be preserved vegetatively, and it should serve as a facility for producing controlled crosses among different genotypes. To date, about 100 different genotypes, covering the species range from Virginia to Texas, have been established. Many of the trees are now flowering, making it possible to produce seed for progeny tests of clones selected for the seed orchards operated by the Tennessee State Division of Forestry. In addition, a number of inter-provenance crosses have been made to determine if wide crosses, such as between trees from Virginia and Texas, will produce trees adapted to our growing conditions (Figure 9). Most of this work has not yet yielded any results; the progenies obtained from controlled crosses are still too young for meaningful evaluation.

The breeding orchard near Knoxville is located about 100 miles north of the species' natural range. Even though the trees grow well in this orchard, they do not flower as early or as abundantly as they would farther South. This observation has some practical implica-

FIGURE 9. Inter-provenance crosses are made in the loblolly pine breeding orchard to determine if wide crosses will produce trees adapted to growing conditions outside the natural range of this species.

tions for organizations interested in establishment of loblolly pine seed orchards for regeneration of land north of the species range. For efficient production of large amounts of seed, such orchards should be located as far south as possible, preferably to within the natural range of loblolly pine.

## CHRISTMAS TREE SPECIES

Since the Christmas tree research program was started in 1960, the number of Tennessee producers has increased from one to more than 50, and the number of trees growing from a few thousand to about 1 million. Producers now know which species will grow best in different locations in the state (4) and production of improved seed has started for several species.

White pine has probably the greatest potential; it has been grown successfully in both East and Middle Tennessee. However, no specific breeding program has been developed to improve the Christmas tree quality of this species. The reason for this apparent neglect is that the characteristics used in the breeding program for white pine timber trees, such as rapid growth and dark green foliage, are also characteristics of great importance for Christmas trees. Thus, the same seed orchards developed for timber production will yield seed needed by our Christmas tree growers.

The other three species with great potential, Fraser fir, Norway spruce, and Scotch pine, have no value as timber in Tennessee and for each species a breeding program had to be developed.

### Fraser Fir

Although Fraser fir has been considered a separate species by both foresters and botanists, the taxonomy of the firs in the Appalachians was so confusing that a study was initiated to determine the natural variation patterns present. For future selection of breeding stock, it would be important to know if distinct ecotypes had developed along the mountain tops in the Southern Appalachians.

From south to north three different "species" were commonly recognized: Fraser fir in North Carolina and Tennessee, intermediate fir in West Virginia, and balsam fir in Pennsylvania and north into Canada. Several stands within the range of each putative species were sampled intensively for characteristics of leaves, cones, and seed (25) as well as wood properties and monoterpene composition in bark blisters (26). For most characteristics, a clinal variation pattern with gradual changes from north to south was evident. The scattered fir stands in the Southern Appalachians are probably remnants of a continuous fir forest. Although it is possible to view these relict populations as potential centers of a new species it is only necessary to recognize one species with three varieties: *Abies balsamea* var. *balsamea* in Pennsylvania, *A. balsamea* var. *phanerolepis* in West Virginia, and *A. balsamea* var. *fraseri* in the southernmost part of the species range.

Some needle characteristics of the southern variety are

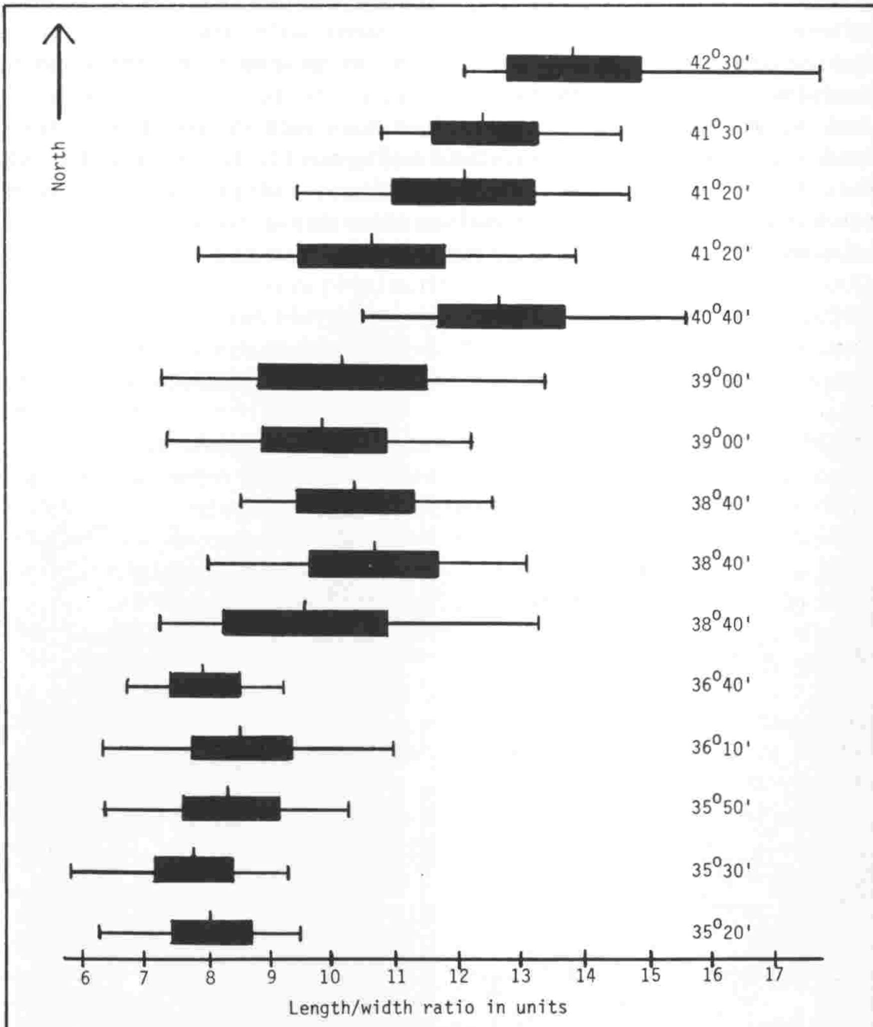


FIGURE 10. Needle shapes (length/width ratio) of native *Abies* in the Southern Appalachians are arranged by stands from south to north. Black squares indicate ± two standard errors; lines represent total range of individual tree values. Needles from the southernmost trees are short and wide but gradually, toward more northern latitudes, needles become narrower and longer.

important to Christmas tree growers. Particularly the greater number of stomatal lines on the under side of the needles gives a silvery appearance; also, needles from the southernmost trees are short and wide but gradually, towards more northern latitudes, needles become narrower and longer. Thus, the needle shape (length/width ratio) shows a very distinct clinal variation pattern (Figure 10). Much of the total variation in needle characteristics is accounted for by this geographic variation pattern; however, variation among individual trees in southern stands is substantial and indicates that significant gains may be obtained by selection of trees with desirable needle characteristics.

A 3-acre seed production area has been established in a mature Fraser fir Christmas tree plantation (Figure 11). Initially, 200 good phenotypes were selected from more than 5,000 planted. Selection was based on characteristics such as stem straightness, balance and fullness of crown, and foliage color.

FIGURE 11. A Fraser fir Christmas tree plantation was heavily rogued to establish this seed production area.

Since the number of buds on the leader may be a good indication of branching density, and the length of the leader is important with regard to shearing requirements and vigor, a small experiment was designed to determine the variation in these characteristics. Total height, leader length, and number of buds on the leader were measured on more than 300 trees in a 4-year-old plantation (27). The relationship between number of buds and leader length or tree height did not account for more than 23 and 10 percent of the variation, respectively. This relative independence of bud number of growth rate suggests that other factors, probably genetic, are responsible for the great variation in number of buds on leaders. With a coefficient of variation for bud number as great as 22 percent, there is a strong suggestion that bud number may be a useful characteristic in a selection program.

Since the use of rooted cuttings for clonal seed orchards may be desirable, a test was run to learn if this technique shows promise for Fraser fir. Cuttings were obtained from three stands located at 4,000, 5,200, and 6,000 feet elevations in the Great Smoky Mountain National Park. Collections were made from five trees in each stand and at three locations within each tree—the lower, middle, and top part of the crown.

Following treatment with hormones (2,000 ppm IBA and NAA) the cuttings were placed in a mist bench with peat moss and sand. By the following spring only 57 of the 675 cuttings had rooted; six of the 15 trees did not produce rooted cuttings at all. Differences among trees in ability to root were highly significant. There were no such clear-cut differences among stands, and although the trees varied in age from 32 to 65 years there was no detectable relationship between age and ability to root. However, highly significant differences in rooting ability were found among cuttings from different locations in the crown; cuttings from the lower portion of the crown had a rooting percent of 16, while those from the middle and top positions had rooting percentages of 8 and 2, respectively.

The poor rooting obtained from cuttings taken from the tops is discouraging since this is the physiologically mature material which is expected to produce seed in a relatively few years following rooting. Another discouraging result was that many of the cuttings continued to grow horizontally, like a branch, for 4 to 6 years after rooting. Even though improved techniques in rooting of cuttings recently have been developed, it appears that other methods, like grafting, may be more suitable for establishing clonal orchards (28).

**Scotch Pine**

*Pinus sylvestris* is native to most of Europe and large parts of Asia. With a distribution from the mild climate of the Mediterranean to harsh growing conditions north of the Arctic Circle, it would be expected that much genetic variation exists within this species. Because of its wide distribution in Europe, its economic importance as a timber tree there, and its common use in reforestation, Scotch pine has probably been studied more intensively than any other forest tree species.

Provenance testing of Scotch pine has been done on an international scale for many years but not a single plantation was established in the South. As a result, Christmas tree growers in Tennessee had no reliable information on suitable provenances. In 1961, seed was obtained from five sources in south and central Europe, 3-year-old seedlings were produced and planted in four locations. The most easterly location was at 4,000 feet elevation in the Blue Ridge Mountains. A second plantation was located at 1,500 feet in the Cumberland Mountains and a third test was established at 2,000 feet elevation on the Cumberland Plateau. The most westerly location was at 1,000 feet elevation on the Eastern Highland Rim. Survival after nine growing seasons was excellent; there were no differences among seed sources in this respect.

Significant differences in height growth were noted following two growing seasons. In general, trees of the two Austrian sources were taller than the others. This difference became more pronounced following five growing seasons, even though annual shearings tend to reduce differences in height growth. At this age one-fourth of the Austrian trees were of sufficient size (at least 5 feet tall) to be marketed, while essentially no trees of the other sources were of marketable size (29). After nine growing seasons, more than one-fourth of the Austrian trees were over 10 feet tall while only an occasional tree of the other sources reached this height. The height difference between the Austrian sources and the Spanish and French was statistically highly significant while there was no difference between the French and Spanish sources. No interaction between seed source and planting site was found; this result indicates that as far as height growth is concerned we do not have to look for specific genetic material for the different growing conditions found in Tennessee.

Short and dark green needles are considered more desirable in Scotch pine than long and yellow ones. Each tree was classified as having short or long needles and dark, light, or yellow-green color. These observations were made from late November to early December, the normal cutting time for Christmas trees in Tennessee. Before November, very few of the trees had a yellow-green color, but by the end of November 35 percent of the Austrian trees had turned this color while only 10 percent of the other trees were classified as having yellow-green foliage. Furthermore, there was some correlation between needle color and needle length; 80 percent of the Austrian trees had long needles but only 20 percent of the trees from the other three sources did. The combination of long needles and yellow-green foliage appears to be particularly undesirable, and since a great number of trees (26-29 percent) of Austrian origin have both these traits, the value of these trees may be limited in our breeding program. However, individual trees of Austrian origin did have green and relatively short needles in addition to the rapid growth rate typical of trees from this source.

The excellent needle characteristics of the Mediterranean sources indicated that additional sources from this region should be tested. A seed source plantation was established with two sources from France and southern sources from Greece, Yugoslavia, and Turkey. Survival was excellent in this Highland Rim plantation; in addition to total height, needle length was measured after 8 years (Table 8). Trees of the two French sources were significantly taller than those of the three South-European sources; also, the needles of the French trees were longer. Considering that the trees of French sources were of marketable size after eight growing seasons while the others would require a 10-year rotation, it is evident that—in spite of their good needle quality—trees of Mediterranean origin will be of limited use in Tennessee. Since data from all provenance tests indicate that trees of French sources have both acceptable growth and needle characteristics, many of the best French phenotypes have been included in our breeding program.

After final evaluation of the provenance tests, some plantations were converted into breeding orchards. All the inferior trees were cut, leaving only the best 10 percent of the original population (Figure 12). These trees were selected on the basis of independent culling levels for growth rate, defects (crooks and forks), needle color,

TABLE 8. Height in feet of five seed sources at the Highland Rim following four and eight growing seasons as well as needle length after 8 years

| Seed source | Lat. N. | Total height in feet 4 yrs. | Total height in feet 8 yrs. | Needle length in cm. at 8 yrs. |
|---|---|---|---|---|
| Haguenau, France | 48°49' | 3.3 | 7.2 | 6.0 |
| Orleans, France | 47°54' | 3.7 | 7.6 | 6.2 |
| Makedonia, Yugoslavia | 41°37' | 2.9 | 6.0 | 5.1 |
| Katerini, Greece | 40°15' | 2.9 | 5.8 | 4.9 |
| Eskisehir, Turkey | 39°46' | 2.6 | 5.4 | 4.7 |

FIGURE 12. The best 10 percent of the trees in a Scotch pine provenance test plantation was selected for breeding purposes.

and Christmas tree grade. Controlled crosses are now made between superior phenotypes of different seed sources. The main objective is to produce a "synthetic variety" by retaining only those genotypes which, after progency testing have been found to combine well with each other.

**Norway Spruce**

Norway spruce (*Picea abies*) is the most important timber species in North and Central Europe and has, because of its good growth and beautiful shape, been extensively planted in the eastern United States. Provenance tests in Europe have determined geographic variation patterns for several characteristics, including growth rate and frost resistance. Typically, the sources from the Arctic Circle are much slower growing than those from Central Europe. This North-South variation pattern tends to be clinal, and in mountainous country a topocline may also be present. Within a given stand there is also a great amount of individual tree variation, both in growth, form, and vigor.

Considering the latitude and climate of Tennessee, no purpose could be seen in testing Norway spruce of north European sources. Rather, the main objective should be to evaluate the magnitude of variation among and within sources from the more southern part of the species range. Such seed source plantations would have to be large enough so that individual trees with desirable Christmas tree characteristics could be selected. Seed for such a test was obtained from five selected stands in Austria and two in Poland and they were planted in four locations in East Tennessee. Trees from all sources survived well, but after ten growing seasons in the field, there were no differences among sources for height growth.

Probably the most important characteristic in breeding of spruce for Christmas trees is needle retention; shedding of Norway spruce needles becomes objectionable after 3 weeks in an indoor environment (30). However, individual trees showed large amounts of variation in needle shedding, indicating a great potential for breeding spruce with better needle retention.

Results of the needle retention study indicated a need for a nondestructive method of sampling to evaluate trees for breeding purposes. Five trees of each of six seed sources of Norway spruce were sampled by removing two branches at each of three crown positions. Seventy-one percent of the total variation in weight loss was associated with samples from within crown position (Table 9), indicating that we must equalize the weight and shape of branches to reduce this error term. Since less than 1 percent of variation was associated with crown position, it would not be necessary to sample at different levels in the crown. The 19 percent of total variation associated with trees within sources indicated that sample branches may be used for evaluation of needle retention of individual trees. A 10 percent variation associated with seed sources suggested that seed source selection for needle retention may be feasible; sources from Poland had the greatest weight loss while Austrian sources had the lowest.

To determine the reliability of branch samples as predictors of needle retention for whole trees, 70 trees were cut; two branches were

TABLE 9. Variation in weight loss of Norway spruce branches after 4 weeks

| Source of variation | Percent | Conclusion |
|---|---|---|
| Seed sources | 10 | Selection among sources is feasible |
| Trees within sources | 19 | Superior phenotypes may be selected |
| Crown position within trees | 1 | Sample from one position only |
| Samples within crown position | 71 | Make all branches same size |

cut from each tree and evaluated for weight loss and appearance after 10 days of drying out. These branch values were correlated with weight loss and appearance of the whole tree after 30 days of drying. Appearance was rated by a preference panel; individual trees and branches were given values from 0 to 3 (Figure 13). Correlation coefficients listed in Table 10 indicate that observations of branches after 10 days give a good estimate of needle-holding capacity of the whole tree. By visually rating two small branches after 10 days of drying, a very inexpensive and fairly reliable estimate of the needle-holding ability was obtained. This simple observation accounted for 60 percent of the variation in weight loss and appearance of whole trees thus providing an efficient means of selecting trees for our breeding program.

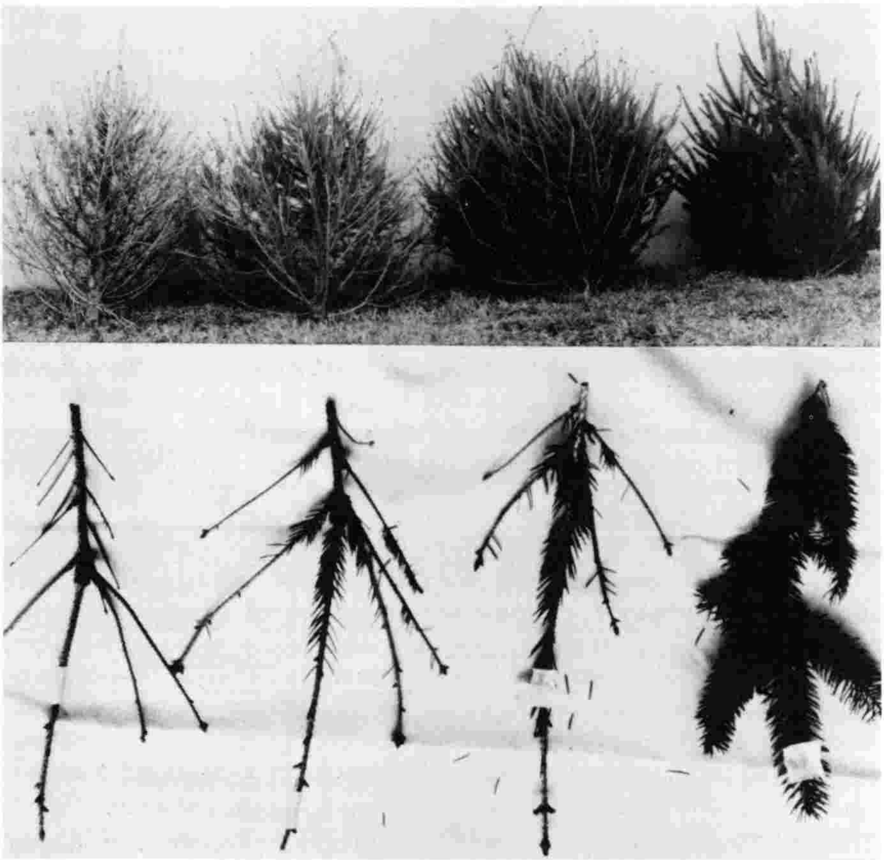

FIGURE 13. Needle-holding of Norway spruce branches after 10 days of drying gives a good estimate of the appearance of whole trees 30 days after cutting. Trees and branches were rated independently from 0 to 3 (left to right).

TABLE 10. Correlation of branch characteristics at 10 days and whole tree characteristics at 30 days for 70 Norway spruce Christmas trees

| Relationship | Correlation coeff. |
|---|---|
| Branch wt. loss X whole tree wt. loss | 0.79 |
| Branch wt. loss X whole tree rating | -0.72 |
| Branch wt. loss X branch rating | -0.91 |
| Branch rating X whole tree wt. loss | -0.79 |
| Branch rating X whole tree rating | 0.77 |
| Whole tree wt. loss X whole tree rating | -0.90 |

This technique was employed in a selection scheme converting the best, most uniform seed source plantation into a seed orchard. Seven hundred trees were, in addition to needle holding, evaluated for height growth, Christmas tree grade, and needle color. The best phenotypes, making up only 10 percent of the original population, were retained; these trees were all above average in height growth, had green or dark green needles, rated U.S. Premium for Christmas tree grade, and scored 3 for needle retention. Since trees from the two Polish sources were significantly poorer in needle retention than trees of Austrian origin, it was decided to remove all Polish trees from the breeding population. Seed or cuttings from this orchard will, after testing to determine the amount of improvement, be released to Christmas tree growers in Tennessee.

## THE AMERICAN CHESTNUT

At one time the Appalachian forests were commonly of the oak-chestnut type. This forest type was dominated by the American chestnut (*Castanea dentata*) which reached 120 feet in height and a diameter of 8 feet. Based on economic factors, chestnut consistently ranked first in importance on many different sites in this region. However, since the introduction of the chestnut blight (*Endothia parasitica*) around the turn of the century, the American chestnut has been practically eliminated from the forest community (1).

Research programs were initiated in hope of getting offspring resistant to the fungus. Interspecific hybridization between *C. dentata* and the resistant oriental species has not yet given successful results as far as producing resistant forest trees. Therefore, an American chestnut intraspecific breeding program was initiated in

1960; this research was based on the hypothesis that crossing surviving American chestnut trees possessing some resistance to the blight fungus may result in occasional progenies with more resistance than either parent. A number of trees that appeared to have some degree of resistance were selected from Tennessee, north Georgia, and western North Carolina (31). Scion wood was collected from these trees and grafted on Asiatic chestnuts. This program is still underway and to date a total of 40 American chestnut trees have been selected. For the past 2 years controlled crosses in the grafted orchard have yielded a good harvest of nuts (Figures 14 and 15) which are now being used in progeny tests (32).

FIGURE 14. Scion wood taken from American chestnut trees selected for apparent resistance to the blight are grafted on Chinese understock. This 10-year-old ramet has an abundant crop of chestnuts.


FIGURE 15. Control-pollinated chestnuts are used in progeny tests to determine if any specific crosses produce trees with a high degree of resistance.

Another approach to chestnut breeding is the irradiation of American chestnut seeds. In the hope of causing a mutation which produces blight resistance, several thousand seeds have been submitted to $^{60}Co$ irradiation (33). So far this approach has been fruitless. Even though some trees grow large enough to produce nuts, they seldom get taller than 10 feet before being killed back by the blight.

A major difficulty confronting breeding programs is the lack of a test to determine blight resistance of young progenies. Natural infection occurs in 5 to 25 years; obviously there is a need for a more satisfactory assay procedure. An investigation of the chemistry of inner bark of chestnut was initiated based on the observation that species of *Castanea* contain high concentrations of phenolic-like compounds and that cankers also contain several of these compounds (34). The inner bark of individual chestnut trees was extracted with four different solvents. Chromatographic comparison of extracts from sound and infected bark from the same tree, and from both American and Chinese chestnut bark, revealed dramatic qualitative and quantitative variation among individual trees. The effect of crude extracts upon the growth of the blight fungus was tested; the chloroform extract of infected inner bark inhibited *in vitro* growth of

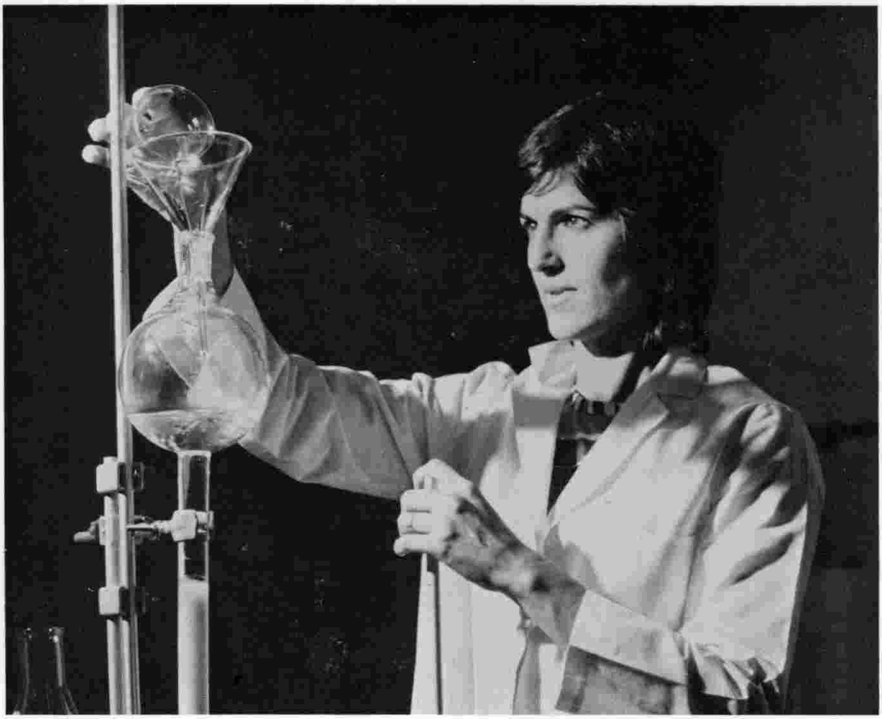

FIGURE 16. Extracts of the inner bark are separated by column chromatography into numerous fractions. Individual fractions are then tested to determine if they inhibit growth of the fungus.

the fungus more than any other extract (35, 36).

The chloroform extract was further investigated and components of the crude extract were separated by column chromatography into 67 fractions (Figure 16). In the development of the column, the polarity of the solvent system was changed by going from pure benzene through diethyl ether, mixtures of diethyl ether-methanol, and benzene-methanol (37). Results of bioassays with the blight fungus indicated the existence of some fungus-inhibiting chemicals (38). Also, the tests indicate that some fractions collected with a mixture of benzene-methanol from the column inhibit fungal growth more than any control (Table 11). The fact that these compounds were eluted with a benzene-methanol mixture indicates that they are highly polar.

Analysis of the fractions by thin layer chromatography (TLC) revealed that these samples were relatively pure. No component was positively idenitified by TLC although many standards, ranging from simple phenols to phenolic acids and flavonoid glycosides, were tested on the same chromatogram. Chromophoric reagents, such as ferric chloride and vanillin, both specific tests for phenolics, gave negative results with all the fractions; therefore the designation of these compounds as phenolics was not possible.

TABLE 11. Effect of five chloroform fractions which significantly reduced *in vitro* growth of *E. parasitica*

| Fraction no. | Mean diameter growth (mm) | % reduction of growth |
|---|---|---|
| 67 | 8.4 | 44 |
| 66 | 9.2 | 39 |
| 64 | 10.9 | 28 |
| 65 | 11.8 | 22 |
| 60 | 11.9 | 21 |
| Mean of 7 controls | 15.11 | |

One interesting fraction (number 66, Table 11) was further investigated for structural analysis. The infrared spectrum revealed an absence of any aromatic compound; no absorption bands were observed at 1,600 $cm^{-1}$ or at 1,500 $cm^{-1}$ where phenyl ring-stretching bands usually are found. Also, C—H stretching was only apparent on the lower frequency side of 3,000 $cm^{-1}$ which indicated that only aliphatic C—H stretching was present and no aromatic C—H stretching occurred. An absorption band observed at 3,500 $cm^{-1}$ indicated that a hydroxyl group is present, another band at 1,730 $cm^{-1}$ indicated the presence of a carbonyl group, probably a carboxylic acid. These observations explain the high polarity of the fraction as indicated by chromatographic data. Proton nuclear magnetic resonance (NMR spectroscopy) confirmed these findings and further indicated a lack of aromatic and olefinic hydrogens. High resolution mass spectral analyses of fraction 66 and its silylated derivatives have been less rewarding; unfortunately, there was no identifiable high mass signal of sufficient strength. However, the mass spectra indicated a long chain hydrocarbon portion.

Determination of a complete structure of fungistatic compounds was hindered by lack of bark extracts. Only small trees, a couple of inches in diameter, had been available. Fortunately, an American chestnut tree 30 years old, 45 feet in height, and 9 inches in diameter was located and permission obtained for its harvest (39). The stem had a dozen cankers giving evidence of repeated infections and ability of this tree to restrict the growth of the fungus. This tree provided 1,000 grams of infected inner bark and 600 grams of sound inner bark. Four grams of crude extract obtained from each sample have been separated into different components by liquid column chromatography. A definite statement regarding the exact chemical nature of fungistatic compounds may be possible following analyses of these components.

The advance of mycelium into the sound inner bark tissue is usually accompanied by a necrosis of tissue in advance of the mycelium. This observation suggests that the fungus produces substances toxic to inner bark tissue; two compounds, diaporthin and skyrin, have been implicated. Culture material of *E. parasitica* of known origins has been collected and is now included in our tests since several strains of the chestnut blight organism may exist (40). Attempts will be made to relate toxins produced by different fungal strains with the killing of inner bark tissue.

The American chestnut may be unique in being the only endangered native plant species adapted to a wide variety of sites and possessing a wide range. Considering the present concern for our environment, it is disturbing that Americans do so little to save a species that has been of such great economic and esthetic importance in their past. At the University of Tennessee we have made some progress in our efforts to save the American chestnut, and we have had some setbacks. But setbacks must be expected in working on a problem that has defied solution for more than half a century. Sufficient progress has been made to encourage initiation of chestnut research programs at other institutions; together we will find an answer to the riddle of the chestnut blight.

## THE FUTURE

Results from applied breeding programs in Tennessee are of greatest value to Tennessee and adjacent states. Typically, information from provenance and progeny testing has most value within the geographic range of the test sites. On the other hand, basic information on heritabilities may be important to breeders in other areas of the country. For species that are commonly planted in foreign countries, this basic information will also be of international importance and our studies of the chemical basis for resistance to chestnut blight will supplement work carried out by foreign researchers.

Although applied breeding work will continue to be of importance in our overall program a greater proportion of this work will in the future be carried out by the Tennessee State Division of Forestry. For example, progeny test plantations for trees selected in the State seed orchard program are now established by the State Division of Forestry. The University of Tennessee tree breeding program will increasingly be more concerned with problems of a basic nature, a development which must be considered compatible with the mission of a large, research-oriented university.

Some of these more basic studies will mainly benefit tree breeding programs in Tennessee and surrounding areas. One such study is concerned with the altitudinal variation in yellow-poplar; seed source tests indicate that yellow-poplars from higher elevations in the mountains have slower growth rates than those from the Valley (see page 16). Seed has been collected from six stands of trees in Tennessee and North Carolina ranging in elevation from 1,600 feet to 3,600 feet. The half-sib families will, under a cooperative regional program, be planted at different elevations in several states to obtain estimates of variation in growth due to genetic differences among stands and trees within stands. Another study of variation in yellow-poplar will be of interest to all tree breeders working with this species. A complete diallel crossing scheme with 12 clones in our breeding orchard is used to provide information about the relative importance of specific *vs.* general combining ability.

This change in emphasis towards more basic research will also be needed to attract the most qualified graduate students. Foreign students are making us aware of problems and opportunities for tree breeding in countries with different growing conditions and economic development; their research must be designed to solve problems relevant to their native countries. In this way our research effort is becoming more international in scope and we may contribute significantly to the worldwide pool of knowledge required for successful tree breeding programs.

International programs in tree breeding and genetics are not limited to training of personnel; the exchange of breeding material on a world-wide scale has greatly increased the potential for improved timber production. More developed countries may in the future derive significant benefits from working with the less developed, often tropical countries by taking advantage of their climatic conditions. Production of southern pine seed in African or South American orchards is one possibility that should be explored, particularly since poor flowering and heavy losses to cone and seed insects make commercial production of genetically improved seed a very costly operation in the United States.

To make a significant contribution to forest genetics and tree breeding, it is essential that continuity is provided in research programs. Frequent changes in emphasis or species to satisfy current demands for more "relevant" research is not conducive to substantial progress in long-term tree breeding. Only gradual changes in emphasis, as suggested by new findings, should be encouraged. Species will be dropped from and added to our program only when dictated by biological or economical circumstances. For such reasons the work with Fraser fir will be discontinued—anticipated gains compared to research costs are not very favorable. On the other hand, preliminary studies with American sycamore (*Platanus occidentalis*) indicate that this species has great potential for fiber production on short rotations and that progress may be made in breeding trees for upland sites.

The large amount of breeding material established for the other seven species in our program should provide significant information and more valuable trees for new plantations in Tennessee. Work should be continued with these species for several years to take full advantage of the progress already made.

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